201-16358A

HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

REVISED TEST PLAN

For

CASHEW NUT SHELL LIQUID

CAS No. 8007-24-7

Submitted to the US EPA

By

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1. Introduction

Cashew nut shell liquid (CNSL) is one of the sources of naturally occurring phenols. It is obtained from the shell of a cashew nut. About 30-35% CNSL is present in the shell, which amounts to approximately 67% of the nut.

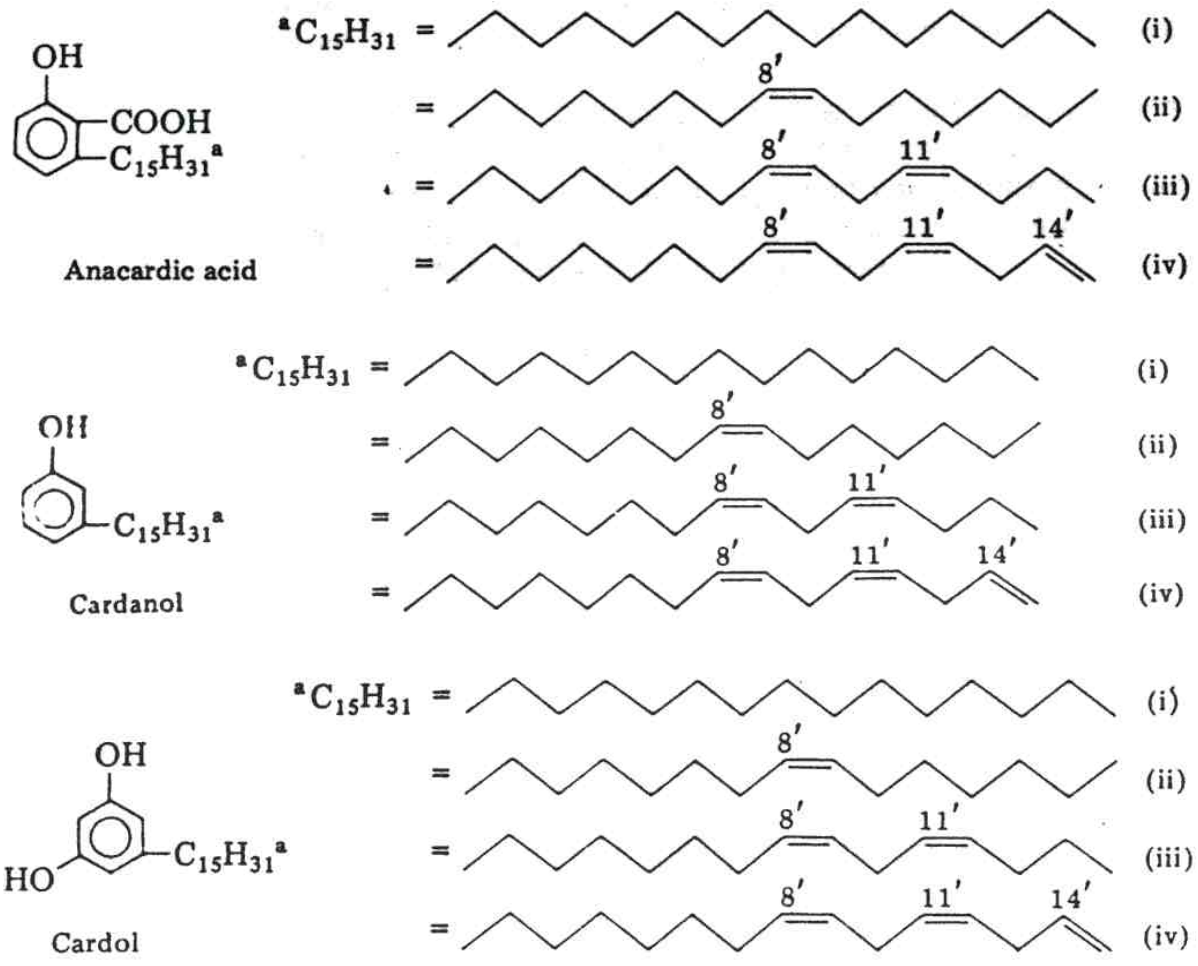
CNSL is traditionally obtained as a by-product during the process of removing the cashew kernel from the nut. The processes used are mainly hot-oil and roasting in which the CNSL oozes out from the shell.

The cashew tree is cultivated globally in tropical areas such as East Africa, South and Central America and the Far East. The world availability of CNSL is in the region of 50,000 tons/year.

1.1 Composition

Natural (i.e. cold, solvent extracted) CNSL contains approximately 70% anacardic acid (Fig 1), 18% cardol, and 5% cardanol, with the remainder being made up of other phenols and less polar substances. As can be seen in Figure 1, anacardic acid, cardanol and cardol consist of mixtures of components having various degrees of unsaturation in the alkyl side-chain.

Figure 1: Structures of Anacardic acid, Cardanol and Cardol



In technical (i.e. heat extracted) CNSL, the heating process leads to decarboxylation of the anacardic acid to form cardanol. Typically, the composition of technical CNSL is approximately 52% cardanol, 10% cardol, 30% polymeric material, with the remainder being made up of other substances.

The technical CNSL is often further processed by distillation at reduced pressure to remove the polymeric material. The composition of distilled technical CNSL is approximately 78% cardanol, 8% cardol, 2% polymeric material, < 1% 2-methyl cardanol, 2.3% heptadecyl homologue triene, 3.8% heptadecyl homologue diene and the remainder other homologous phenols.

Table 1 summarises the composition of typical batches of technical and distilled technical grades of CNSL.

Table 1: Composition of typical batches of Technical and Distilled CNSL

	Cardanol	Cardanol monoene	Cardanol diene	Cardanol triene	Cardol diene	Cardol triene	Polymer	2-methyl cardanol	C17 triene	C17 diene	Unidentified phenols
T-CNSL	0.06	17.10	10.78	24.42	2.36	7.50	30.6				5.83
D-CNSL	-	25.9	16.2	35.8	2.04	5.90	2.5	0.60	2.27	3.75	5.04
AT-CNSL	0.09	24.7	15.6	35.3	3.41	10.8	-				8.42

T-CNSL = Technical grade, D-CNSL = Distilled grade, AT-CNSL = Technical grade component percentages adjusted for removal of polymer.

1.2 Commercial Applications

CNSL resins have been used extensively in the manufacture of friction-resistant components in applications such as brake and clutch linings. These resins are used as binders for friction ingredients and also as friction ingredients themselves in the form of fine dusts obtained from the completely cured resins.

CNSL-aldehyde condensation products and CNSL-based phenolic resins are used in applications such as surface coatings, adhesives, varnishes and paints. Various polyamines synthesised from CNSL or cardanol are used as curing agents for epoxy resins.

CNSL and its derivatives have been used as antioxidants, plasticisers and processing aids for rubber compounds and modifiers for plastic materials. Resins based on the reaction products of cardanol phenol and formaldehyde are used to improve the resistance of rubber articles to cracking and ozone. CNSL, cardanol and cardol are all used to provide oxidative resistance to sulfur-cured natural rubber products. Cardanol, CNSL or sulfurated CNSL is added to rubber gum stock or nitrile rubber to improve the processability, mechanical properties and resistance to crack and cut properties of the vulcanisates.

A number of products based on CNSL are used as antioxidants, stabilisers and demulsifiers for petroleum products. Metal xanthates of partially hydrogenated, sulfurised cardanol is used to lower the pour point of lubricating oils as well as acting as antioxidant and anticorrosive properties. Soluble metal derivatives of CNSL are used to improve the resistance to oxidation and sludge formation of lubricating oils. Oxidised CNSL and its derivatives are used as demulsifying agents for water in oil type petroleum emulsions.

1.3 Worker/consumer exposure

Only large industrial manufacturers use CNSL. There are no direct consumer applications and therefore no direct sales to the general public. The most likely source of consumer exposure to CNSL is through contact with contaminated nuts, although reports of adverse effects arising from such contact appear to be rare.

Exposure of workers to CNSL during production is most likely to occur during removal of the kernels from the nuts, after processing to remove the CNSL, especially in countries where the shelling has not been mechanised. Exposure to CNSL can lead to sensitisation and dermatitis. Workers in these countries are given some protection to exposure through the use of barrier creams.

Workers involved in the further processing the CNSL to manufacture commercial products are likely to have minimal exposure to the CNSL as it is expected that good industrial hygiene practices will be followed and personal protective equipment worn to minimise exposure.

2. Rationale for Selection of Compounds for testing

Distilled Technical CNSL has been selected as the most suitable substance for testing to fulfil the requirements of the HPV Challenge Program. This is because it is possible to obtain distilled CNSL to a more consistent specification than the Technical CNSL and the distilled grades are becoming more important industrially than the crude technical grade material. It is also believed that since all of the substances in CNSL are based on phenols having various degrees of unsaturation in the side chain, they meet the EPA's criterion of using the 'family approach', thus the toxicological properties of the Distilled CNSL will be representative of the properties of the Technical CNSL.

3. Review of Existing Data and Development of Test Plan

Cardolite Corporation has undertaken a comprehensive evaluation of all relevant data on the SIDS endpoints of concern for Cashew Nut Shell Liquid.

The availability of the data on the specific SIDS endpoints is summarized in Table 2. Table 2 also shows data gaps that will be filled by additional testing.

Table 2: Available Adequate Data and Proposed Testing on Cashew Nut Shell Liquid*

CAS No. 8007-24-7	Information Available?	GLP	OECD Study?	Other Study?	Estimation Method?	Acceptable?	SIDS Testing required?
	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Physicochemical							
Vapour Pressure	Y	Y	Y	-	N	Y	N
Partition Coefficient (Kow)	Y	Y	Y	-	N	Y	N
Water solubility	Y	Y	Y	-	N	Y	N
Environmental Fate & Pathway							
Photodegradation	Y	N	N	-	Y	Y	N
Transport and distribution	Y	N	N	-	Y	Y	N
Biodegradation	Y	Y	Y	-	-	Y	N
Ecotoxicology							
Acute Fish	Y	-	-	-	Y	Y	N

Acute Daphnia	Y	-	-	-	Y	Y	N
Acute Algae	Y	-	-	-	Y	Y	N
Toxicology							
Acute Oral	Y	Y	-	-	-	Y-	N**
Repeat Dose toxicity	Y	Y	Y	-	-	Y	N
Genetic toxicity – Gene mutation	Y	Y	Y	-	-	Y	N
Genetic toxicity – Chromosome aberration	Y	Y	Y	-	-	Y	N
Reproductive toxicity	Y	Y	Y	-	-	Y	N
Developmental toxicity/teratogenicity	Y	Y	Y	-	-	Y	N

* No testing has been conducted for melting point, boiling point or hydrolysis.
** This endpoint has been filled using information from the OECD 422 range-finding study

A. Evaluation of Existing Physicochemical Data

1. Melting Point

Melting point will not be determined, as the substance is a liquid under ambient conditions.

2. Boiling Point

A boiling point at ambient pressure has no significance, as the substance will be subject to thermal polymerization and decomposition before boiling. Accordingly, measurement of this property is inappropriate for this substance.

3. Vapor Pressure

The vapour pressure of cashew nutshell liquid, determined in a GLP study using a vapour pressure balance method (OECD TG104, OPPTS 830.7950), is 5.0×10^{-5} Pa at 25°C.

4. Water solubility

The water solubility of cashew nutshell liquid, determined in a GLP study using the shake flask method (OECD TG 105, OPPTS 830.7840), is 3.04×10^{-4} g/L of solution at 20°C.

5. Partition Coefficient

The partition coefficient of cashew nutshell liquid, determined in a GLP study using the HPLC method (OECD TG117, OPPTS 830.7570), is log Pow > 6.20.

Summary of Physicochemical Properties Testing:

The water solubility was determined to be 3.04×10^{-4} g/L of solution at 20°C in a guideline study (OECD TG 105, OPPTS 830.7840) performed to GLP. The vapour pressure was determined to be 5.0×10^{-5} Pa at 25°C in a guideline study (OECD TG104, OPPTS 830.7950) performed to GLP. The partition coefficient of all components of cashew nutshell liquid was determined to be log Pow > 6.20 in a guideline study (OECD TG 117, OPPTS 830.7570) performed to GLP. Tests for melting point and boiling point are not applicable to these substances.

B. Evaluation of Existing Environmental Fate Data

1. Biodegradation

Distilled CNSL has been shown to be biodegradable when tested using OECD Method 301D (96% degradation after 28 days) in a GLP study.

2. Hydrolysis

Hydrolysis as a function of pH is used to assess the stability of a substance in water. Hydrolysis is a reaction in which a water molecule (or hydroxide ion) substitutes for another atom or group of atoms present in an organic molecule. None of the major components of CNSL contain a functional group that would be susceptible to hydrolysis. Therefore, hydrolysis need not be measured.

In addition, low water solubility often limits the ability to determine hydrolysis as a function of pH. The water solubility of cashew nutshell liquid was determined to be 3.04×10^{-4} g/L. Therefore, these materials are expected to be stable in water and it would be unnecessary to attempt to measure the products of hydrolysis.

3. Photodegradation

Indirect photo-oxidation by hydroxy radicals (1500000 molecule/cm^3) is predicted to occur with a half-life estimated at 0.351-1.254 hrs (calculated using AOPWIN v1.91 at 25 °C, rate constant, 102.3748 – 365.8109 E-12 cm^3/molecule/sec, 12-hour day).

4. Transport and Distribution between Environmental Compartments

Based on Epiwin V3.12 Level III Fugacity Model estimations, the major components of cashew nutshell liquid will distribute mainly to soil (37.3 - 77.7%) if released to the air compartment, almost exclusively to sediment (94.8 – 97.3%) if released to water, exclusively to soil if released to soil and mainly to sediment (66.9 – 69.9%) if released simultaneously to all air, soil and water compartments.

Summary of Environmental Fate Testing:

Distilled CNSL has been shown to be biodegradable when tested using OECD Method 302D (96% degradation after 28 days) in a GLP study. None of the major components of CNSL contain a functional group that would be susceptible to hydrolysis. Indirect photo-oxidation by hydroxy radicals (1500000 molecule/cm3) is predicted to occur with a half-life estimated at 0.351-1.254 hrs (calculated using AOPWIN v1.91 at 25 °C, rate constant, 102.3748 – 365.8109 E-12 cm3/molecule/sec, 12-hour day). Based on Epiwin V3.12 Level III Fugacity Model estimations, the major components of cashew nutshell liquid will distribute mainly to soil (37.3 - 77.7%) if released to the air compartment, almost exclusively to sediment (94.8 – 97.3%) if released to water, exclusively to soil if released to soil and mainly to sediment (66.9 – 69.9%) if released simultaneously to all air, soil and water compartments.

C. Evaluation of Existing Ecotoxicity Data

The basic ecotoxicity data that are part of the HPV program include acute toxicity to fish, daphnia and algae. Predicted values for the 2 main chemical components, and their

analogs, of CNSL have been obtained using ECOSAR v 0.99e [(1)]. These values predict that CNSL will be toxic in the aquatic environment, therefore it is unnecessary to generate experimental data. This is further supported by the low solubility of CNSL, which is $3.04x10^{-4}$ g/L of solution at 20°C, making analytical monitoring of the tests very difficult and making the tests themselves unlikely to determine any more useful or accurate estimates of toxicity than the estimations.

Summary of Ecotoxicity Testing:

The 2 main components of CNSL, and their analogs, are predicted to be acutely toxic to fish (LC_{50} (96h) < $11x10^{-3}$ mg/l), daphnia (LC_{50} (48h) < $66x10^{-3}$ mg/l) and algae (EC_{50} (96h) < $1x10^{-3}$ mg/l).

D. Evaluation of Existing Human Health Effects Data and Proposed Testing

1. Acute Oral Toxicity

In a 14-day range-finding study, performed as part of the combined repeat dose reproduction/developmental toxicity screening test (OECD TG 422) no mortality or clinical signs of toxicity were observed at the maximum dose of 1000 mg/kg bw/day. It is concluded that the LD_{50} for acute oral toxicity is likely to be greater than 1000 mg/kg bw.

2. Repeat Dose Toxicity

In a combined repeat dose reproduction/developmental toxicity screening study (OECD 422, GLP), male and female rats were dosed by gavage daily with CNSL in arachis oil at 0, 15, 150 or 1000 mg/kg bw/day for up to 49 days. There were no treatment-related deaths during the study. A slightly reduced bodyweight gain was observed for 1000 mg/kg/day males during the first two weeks of the study. Reduced bodyweight gain was also observed for 1000 mg/kg/day females during the later stages of the gestation period. No adverse effect on dietary intake or food efficiency was detected. No intergroup differences in water consumption were detected. Increased salivation was detected prior to dosing and up to five hours after dosing for animals of either sex treated with 1000 mg/kg/day from Day 9 onwards. An isolated incident of increased salivation was detected up to one hour after dosing for one male treated with 150 mg/kg/day on Day 8 only. One female treated with 150 mg/kg/day developed hunched posture, pilo-erection, tiptoe gait, red/brown staining around the mouth, laboured respiration and pallor of the extremities on Day 5 and was subsequently terminated due to the severity of these observations. On Day 39 of the study (gestation Day 21), one female treated with 1000 mg/kg/day showed staining around the vagina, hunched posture, tiptoe gait, pilo-erection, pallor of the extremities and red/brown staining around the snout. This animal had given birth to a total of sixteen pups, twelve of which were found dead. This animal, together with the live pups were subsequently terminated on humane grounds. No such observations were observed for animals of either sex treated with 15 mg/kg/day. Increased salivation was detected for individual animals of either sex treated with 1000 mg/kg/day during the Week 3 and Week 4 assessments. This observation was also observed for two 1000 mg/kg/day males during the post-mating assessments and for one 1000 mg/kg/day female during the Day 4 *post partum* assessments. No such effects were detected for animals of either sex treated with 150 or 15 mg/kg/day. All remaining inter and intra group differences in urination, defecation and transfer arousal scores were considered to be a result of normal variation for rats of the strain and age used and were of no toxicological importance. There were no treatment-related changes in the functional performance parameters measured or in

sensory reactivity. Elevated platelet counts were observed for males treated with 1000 mg/kg/day during the pre-mating and pre-termination investigations. Elevated haemoglobin, erythrocyte and haematocrit counts were also evident for 1000 mg/kg/day males during the pre-mating and pre-termination investigations. Males treated with 1000 mg/kg/day also displayed elevated mean cell volume (MCV) and a reduction in mean cell haemoglobin concentration (MCHC) during the pre-mating and pre-termination investigations. An increase in MCV was also seen for 1000 mg/kg/day females during pre-mating and terminal assessments. Although the aetiology of these changes is unclear, the level of significance achieved for haematocrit and MCHC cannot be disregarded. No treatment-related effects were detected for animals of either sex treated with 150 or 15 mg/kg/day. Elevated aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and inorganic phosphorus were detected for animals of either sex with 1000 mg/kg/day during blood chemical investigations. A reduction in cholesterol and elevated bilirubin levels were also detected, with effects more prominent in the males, which also showed a slight increase in plasma urea, reduction in glucose levels and elevated albumin/globulin ratios during the pre-termination investigations. No such observations were detected for animals of either sex treated with 150 or 15 mg/kg/day. No treatment-related microscopic abnormalities were detected at terminal kill. Females treated with 1000 mg/kg/day showed a statistically significant increase in relative liver weight. Absolute liver weight was also elevated, but statistical significance was not achieved. An increase in absolute and relative adrenal weight was also observed for females. No such observations were detected for 1000 mg/kg/day males or for animals of either sex treated with 150 or 15 mg/kg/day. The following treatment related changes were found: Lungs - Groups of alveolar macrophages were seen with a higher incidence for females treated with 1000 mg//kg/day only; Mesenteric lymph nodes - A higher incidence of sinus histiocytosis and/or foamy histiocytes, was observed in relation to treatment for animals treated with 1000 mg/kg/day but not at any other dose level; Stomach - Hyperkeratosis, frequently associated with acanthosis, was seen in the forestomach of animals of either sex treated with 1000 mg/kg/day. Focal ulceration of the forestomach epithelium was also seen for one high dose female. Treatment-related changes did not extend to the remaining treatment groups; Duodenum - Mucosal hypertrophy was seen in three males treated with 1000 mg/kg/day. A low incidence of mucosal hypertrophy was observed in all groups of females. The ‘No Observed Effect Level’ (NOEL) for systemic toxicity was therefore considered to be 150 mg/kg/day.

3. Genotoxicity

Distilled CNSL has been tested for potential genotoxicity in the Ames Salmonella assay (strains TA1535, TA1537, TA1538, TA98 and TA100), an in vitro chromosome aberration test in human lymphocytes, and an in vitro HGPRT forward mutation assay using a Chinese Hamster Ovary cell line. None of these test systems showed any indication of genotoxicity. All three studies were conducted under GLP.

4. Reproductive and Developmental Toxicity

In a combined repeat dose reproduction/developmental toxicity screening study (OECD 422, GLP), male and female rats were dosed by gavage daily with CNSL in arachis oil at 0, 15, 150 or 1000 mg/kg bw/day from 14 days prior to mating up to Day 4 post partum. Parental effects are described in the repeat dose toxicity section. There were no treatment-related intergroup differences in litter sizes. The intergroup variability in live litter size at birth and through early lactation was not reflected in any difference in percentile pre and post implantation loss and there was no significant increase in post partum

invariability. There was no treatment-related difference in live birth index or viability index after 4 days and no toxicologically significant clinical findings were observed in offspring. No treatment-related macroscopic abnormalities were detected at terminal kill. No treatment-related effects were detected in postnatal growth. A statistically significant reduction in litter weight gain was detected in offspring of females treated with 1000 mg/kg/day in comparison to controls; however this was most probably a consequence of smaller litter numbers in the high dose and in the absence of intergroup differences in mean pup weight was not considered a reproductive effect of treatment. The NOEL for reproductive and developmental toxicity was considered to be 1000 mg/kg/day.

5. Skin Sensitisation

This non-SIDS endpoint has been evaluated using distilled CNSL in a Guinea pig maximisation test (OECD 406). The test substance produced a 70% (14/20) sensitisation rate and was classified as a strong sensitiser.

6. Oestrogenic Activity

This non-SIDS endpoint has been evaluated using two grades of distilled CNSL in a recombinant yeast screen assay. The two distillates showed no oestrogenic activity under the conditions of the test.

Summary of Human Health Effects Testing:

Results from a 14-day repeat dose range-finding study indicate that the LD50 for acute oral toxicity is likely to be greater than 1000 mg/kg bw. In a combined repeat dose with reproduction/developmental toxicity study (OECD 422, GLP), the oral administration of CNSL to rats by gavage at the maximum dose level of 1000 mg/kg/day resulted in treatment-related systemic changes in the lungs, mesenteric lymph node, stomach, and duodenum. The 'No Observed Effect Level' (NOEL) for systemic toxicity was therefore considered to be 150 mg/kg/day. No effect of treatment was detected on reproduction or offspring development, therefore the NOEL for reproductive and developmental toxicity was considered to be 1000 mg/kg/day. Distilled CNSL has been tested and found negative in three in vitro genotoxicity assays therefore no additional testing for this endpoint will be undertaken. Distilled CNSL has been shown to be a strong skin sensitiser in guinea pigs and to have no oestrogenic activity when tested in a recombinant yeast screen assay.

4. Evaluation of Data for Quality and Acceptability

The collected data were reviewed for quality and acceptability following the general US EPA guidance (3) and the systematic approach described by Klimisch et al (4). These methods include consideration of the reliability, relevance and adequacy of the data in evaluating their usefulness for hazard assessment purposes. This scoring system was only applied to ecotoxicology and human health endpoint studies per EPA recommendation (5). The codification described by Klimisch specifies four categories of reliability for describing data adequacy. These are:

(1) Reliable without restriction: Includes studies or data complying with Good Laboratory Practice (GLP) procedures, or with valid and/or internationally accepted testing guidelines, or in which the test parameters are documented and comparable to these guidelines.

(2) Reliable with Restrictions: Includes studies or data in which test parameters are documented but vary slightly from testing guidelines.

(3) Not Reliable: Includes studies or data in which there are interferences, or that use non-relevant organisms or exposure routes, or which were carried out using unacceptable methods, or where documentation is insufficient.

(4) Not Assignable: Includes studies or data in which insufficient detail is reported to assign a rating, e.g. listed in abstracts or secondary literature.

7. References

1. ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

2. USEPA (1998). Guidance for Meeting the SIDS Requirements (The SIDS Guide). Guidance for the HPV Challenge Program. Dated 11/2/98.

3. Klimisch, H.-J., et al (1997). A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regul. Toxicol. Pharmacol. 25:1-5

4. USEPA (1999). Determining the Adequacy of Existing Data. Guidance for the HPV Challenge Program. Draft dated 2/10/99.